31.8.2015

MONDAY, 8/31

Anna purified Amph +term (no linker) with PCR purification kit according to the kit's protocol.
48,1ng/µl, A280/260: 1,81

Restrictions for AH045 (EcoRI + SpeI), Amph+term (no linker) (XbaI + PstI) and linear pSB1C3 (EcoRI + PstI). 3.1 buffer.

AH045
- 4,4µl DNA
- 17,1µl H2O
- 2,5µl 3.1 buffer
- 0,5 µl EcoRI
- 0,5µl SpeI

Amph+term (no linker)
- 5,2µl DNA
- 16,3µl H2O
- 2,5µl 3.1 buffer
- 0,5 µl XbaI
- 0,5µl PstI

Linear pSB1C3
- 10µl DNA
- 11,5µl H2O
- 2,5µl 3.1 buffer
- 0,5 µl EcoRI
- 0,5µl PstI

-> +37C,90min

Running another SDS-PAGE for CAR samples:
Spinned the samples (made 25.8) for 1 min, 14000 rpm. Used the gel made 25.8. Made sample dilutions: 10 ul original sample to 20 ul water. Didn't dilute control sample. Pipetted the samples (30 µl each, 20 µl control) to the wells. Used 7 ul Bio-Rad Precision Plus Protein Dual color standard. Pipetting order:

1. Marker
2. CAR in pSB1C3 gibson
3. CAR gibson 2 (in AH043)
4. CAR gibson 3 (in AH043)
5. CAR gibson 4 (in AH043)
6. CAR gibson 7 (in AH043)
7. Control: DEL1 cells without plasmid

Ran the gel first in 90 V and rinsed to 120 V when the marker was separated a bit. Total time ran: 70 min.

Staining the gel:
Stained the gel in Coomassie Brilliant Blue for 30 min with shaking. Let the gel sit in destaining buffer o/n.

O/n cultures of AtoB and CenA gibson colonies:
Made 5 cultures of Atob and 5 of CenA (same colonies analyzed with colony PCR last week):
- 2 ul LB
- 2 ul chloramphenicol stock (stock concentration 34 mg/ml)
- incubated in 37 C with shaking

Ligated twice restricted AH045 with Amph (no linker) and pSB1C3 following the T4 enzyme kit protocol. Using molar rate of 2:1, there were: AH045 0,2 ul, Amph 2,2 ul and pSB1C3 3 ul. And using molar rate of 3:1: AH045 0,3 ul, Amph 3,3 ul and pSB1C3 3 ul.

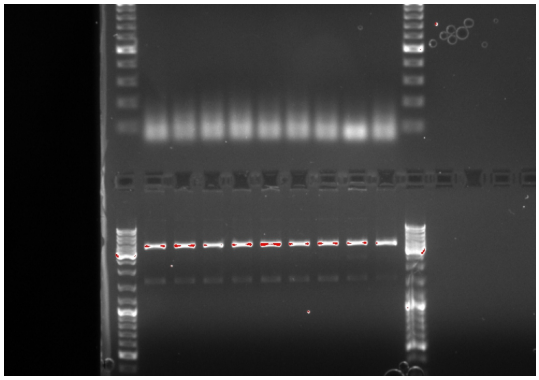
Transformed the ligations to Top10 using 5 µl of each ligation following the protocol. Plated 50 µl and 150 µl from each tube to CAM plates. Also did transformation efficiency test to new CHEM competent cells transfoming #37 1 µl to new Top10 cells.

Did o/n cultures of GFP+Amph+AH045 of colonies which emitted green colour in UV (2 tubes) using 2 µl AMP in 2 ml LB.

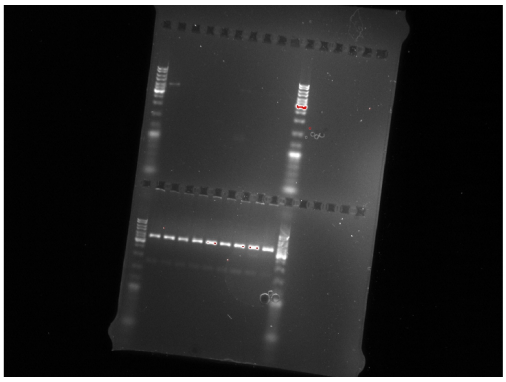
Restricted Car Amph using X & P following mostly the protocol. However, the incubation was 1,5 h.

Made a 1,3 % agarose gel with EtBr. Did run restrictions and colony PCR reactions of Car Amph in the gel for 40 min with 100 V.
Pipeting order was:
I 1. ladder 2 µl 2.-9. Car Amph cPCR 6 ul 10. ladder 2µul
II 1. ladder 2µl 2.-9. Car Amph rest 6 ul 10. ladder 2 µl

Geldoc_2015-08-31_12hr_49min_cPCRrestofCarAmph.jpg



Geldoc_2015-09-01_10hr_22min_onrestCAR_AMPH.jpg



Result: There were not Car Amph insert in any selected colony.

1.9.2015

TUESDAY, 9/1

Petra

Miniprepped yesterday's AtoB & CenA o/n cultures using NucleoSpin Plasmid EasyPure kit.

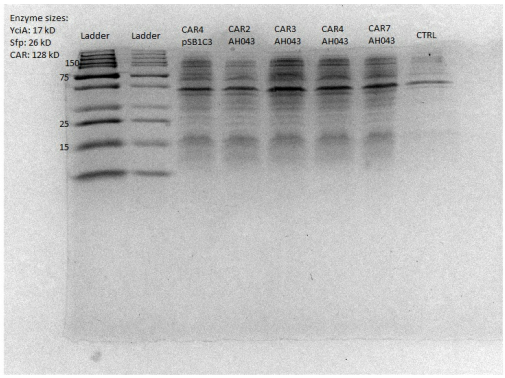
Nanodrop results are in Table 1.

Table1

Construct	Concentration (ng/µl)	A260/A280	Amount pipetted for 250 ng (µl)	Water added to restriction (µl)
AtoB 1 in pSB1C3	53,4	1,91	4,7	16,6
AtoB 2 in pSB1C3	57,5	1,93	4,4	16,9
AtoB 3 in pSB1C3	51,1	1,94	4,9	16,4
AtoB 4 in pSB1C3	37,5	1,94	6,7	14,6
AtoB 5 in pSB1C3	52,4	1,92	4,8	16,5
CenA 1 in pSB1C3	44,2	1,88	5,7	15,6
CenA 2 in pSB1C3	51,1	1,77	4,9	16,4
CenA 3 in pSB1C3	43,8	1,95	5,7	15,6
CenA 4 in pSB1C3	43,7	1,91	5,7	15,6
CenA 5 in pSB1C3	6,2	1,90	40,3	2,6

Took a picture of CAR SDS-PAGE gel that was left to destaining buffer overnight.

SDS-page_CAR_Geldoc_2015-09-01_13hr_47min_SDS-page.jpg



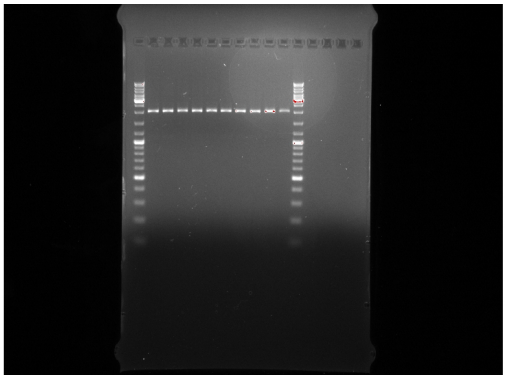
The picture is clearer than the previous gel picture, but unfortunately we can't tell whether some of the enzymes are overexpressed compared to the control sample. Result: SDS-PAGE analysis of CAR construct was unsuccesful.

Restricted miniprepped AtoB and CenA constructs with EcoRI and PstI. Pipetted DNA and water acccording to Table 1. Added 2,5 ul NeBuffer 3.1. Used 0,7 ul EcoRI and 0,5 ul PstI.
Restriction for CenA 5 in pSB1C3: due to the low concentration pipetted DNA and water according to the Table 1, added 5 ul NeBuffer 3.1. Used 1,4 ul EcoRI and 1 ul PstI. Total reaction volume: 50 ul.
Incubated restriction reactions in 37 C for 1h 30min.

Made a 1,2 % agarose gel with ETBR. Pipetted 5 µl samples of restriction reactions on gel with 1 µl LD. Used GeneRuler ladder. Ran the gel for 40 min, 120V. Pipetting order:

1. Ladder
2. AtoB 1 in pSB1C3
3. AtoB 2 in pSB1C3
4. AtoB 3 in pSB1C3
5. AtoB 4 in pSB1C3
6. AtoB 5 in pSB1C3
7. CenA 1 in pSB1C3
8. CenA 2 in pSB1C3
9. CenA 3 in pSB1C3
10. CenA 4 in pSB1C3
11. CenA 5 in pSB1C3
12. Ladder

Geldoc_2015-09-01_16hr_53min.jpg



According to the gel picture none of the samples contain the right insert. The band seen in every row is linearized backbone (2070 bp).

Yesterday's transformation of AH45+GFP+pSB1C3 was successful, so did o/n cultures of AH045+Amph+pSB1C3 from several colonies using 2 ul CAM in 2 ml LB.

Also transformation efficiency test results OK, so we can use the new Top10 CHEM competent cells.

Miniprepped yesterday's o/n cultures of GFP+Amph+linker following the kit protocol.
NanoDrop result:

Table2

Sample	DNA (ng/ul)	A260/A280
GFP+Amph 1	177,5	1,84
GFP+Amph 2	161,9	1,85

Minipreps were stored in -20 C.

Juuso:
did transformation work for Tamannae as she had to leave, here are my notes:

16:15
+50 µl TOP10 (blue marking) to 2 ml eppendorfs marked "trans 2:1" and "trans 3:1"
+5 µl of respective ligation mix prepared by tamannae to the tubes above (2:1 -> 2:1 and 3:1 -> 3:1)
- mixes used: "31.8. lig amph 2:1" and "31.8. lig amph 3:1"
+50 µl TOP10 (black marking) to 2 ml eppendorf "TE 31.8"
+1 µl mix "#37 1:50" to the "TE 31.8" eppendorf

-> incubated the three tubes 30 min on ice

16:35 fetched 6 chloramphenicol plates from cold room, named them
"CAM 31.8. trans. lig. amph. 2:1 50 µl JR -> TA"
"CAM 31.8. trans. lig. amph. 2:1 150 µl JR -> TA"
"CAM 31.8. trans. lig. amph. 3:1 50 µl JR -> TA"
"CAM 31.8. trans. lig. amph. 3:1 150 µl JR -> TA"
"CAM 31.8. TE 50 µl JR -> TA"
"CAM 31.8. TE 150 µl JR -> TA"

16:45 heat shocked cells (3 tubes from above) 60 sec 42C heat block
-> on ice 5 min 16:46-16:50

16:50 added 200 µl SOC ("iGEM SOC 22.6.")
-> incubation 37C with shaking 200 rpm 55 min (gene lab incubator)

17:45 plated transformants on the aforementioned plates, 50 or 150 µl per plate, let dry for 10 min
-> 37 C o/n

2.9.2015

WEDNESDAY, 9/2

Petra

Since the screening of AtoB and CenA colonies was unsuccesful decided to screen more colonies. Chose 5 colonies from AtoB plates and 5 colonies from CenA plates to screen with KAPA colony PCR. Named the colonies with numbers 6-10 (AtoB 6-10 & CenA 6-10).

11x reaction mix without template DNA (253 µl)
55 µl 5x KAPAHiFi Fidelity Buffer
8,25 µl dNTP mix
8,25 µl 10 µM P001
8,25 µl 10 µM P011
5,5 µl KAPAHiFi HotStart DNA Polymerase
167,75 µl water

Pipetted 23 µl reaction mix to 10 PCR tubes. Added 2 ul template DNA to each tube.

KAPA colony PCR for CenA and AtoB from Gibson assembly: PCR program
95° *C - 3 min*
98° *C - 20 sec*
64° *C - 15 sec*
72° *C - 3,5 min*
72° *C - 4 min*
4° *C - forever*
Repeated underlined cycles 25 times

Plated all the colonies used for colony PCR (AtoB 6-10 and CenA 6-10) on a fresh cloramphenicol plate and incubated in 37 C overnight.

Miniprepped Amph (no linker) following the kit protocol.
NanoDrop results:

Table1

Sample	DNA (ng/ul)	A260/A280
Amph 1	72,9	1,82
Amph 2	40,1	1,87
Amph 3	64,0	1,87
Amph 4	437,1	1,83
Amph 5	30,0	1,94
Amph 6	59,8	1,87
Amph 7	56.4	1,87
Amph 8	83,6	1,84
Amph 9	61,0	1,87
Amph 10	43,3	1,88

Restricted AH043 with X&S following mostly the protocol. However, restricted 500 ng and the incubation was 1,5 h.

Because we didn't get Car Amph constructed, we tried to create again it using NEB Hifi Assembly kit (gibson).
In the reaction mix, there were:
Car part 1 1,12 ul
Car part 2 0,3 ul
Car amph part 3 0,82 ul
Car amph part 4 0,4 ul
AH043 (rest.) 6,24 ul
Buffer 8,88 ul

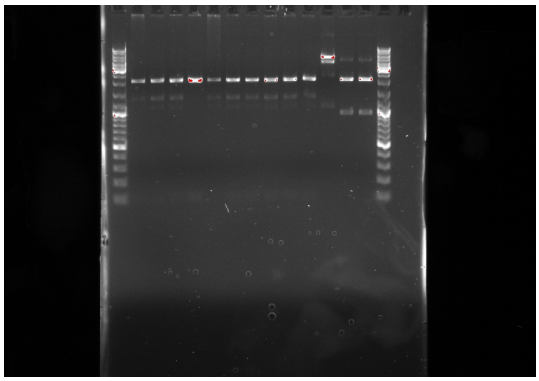
Transformed 4 ul of Car Amph (gibson) to NEB CHEM competent cells following the protocol. Plated 10 ul, 50 ul and 150 ul to AMP plates.

Restricted GFP+Amph+linker and Amph with E&P following mostly the protocol. However, the incubation was 1,5 h.

Made a 1,3 % agarose gel with EtBr. Restricted Amph, AH043 and GFP+Amph were run in the gel for 40 min with 100 V. Pipeting order was:

1. ladder 2 ul 2.-11. Amph 1-10 6 ul 12. AH043 6 ul 13.-14. GFP+Amph 6 ul 15. ladder 2 ul

Geldoc_2015-09-02_15hr_28min_AmphAH043GFPAmph2.jpg



Result: Expected to get only 2 bands in each lane, so it is difficult to say if we have correct constructs or not. It seems that Amph parts in the both constructs are not correct ones. However, we do know that after fusing AH045+GFP with Amph+linker caused GFP to work (green colonies in UV), so GFP got somehow stop codon in the correct reading frame. Even biobrick backbone AH043 was cut several pieces so it might be that enzymes or used buffer (3.1) are contaminated.

Because of the gel result, picked new colonies of Amph and did o/n cultures of them in 2 ml Lb with 2 ul CAM (9 tubes).

3.9.2015

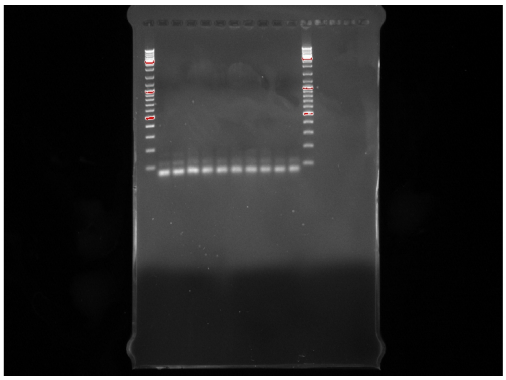
THURSDAY, 9/3

Petra

Running yesterday's colony PCR reactions on gel:
Made a 1,2 % agarose gel with ETBR. Ran 5 µl sample of each colony PCR reaction (AtoB 6-10 and CenA 6-10) on gel. Added 1 µl LD to each sample. Used GeneRuler ladder. Ran the gel for 40 min, 120V. Pipetting order:

1. Ladder
2. AtoB Gibson 6
3. AtoB Gibson 7
4. AtoB Gibson 8
5. AtoB Gibson 9
6. AtoB Gibson 10
7. CenA Gibson 6
8. CenA Gibson 7
9. CenA Gibson 8
10. CenA Gibson 9
11. CenA Gibson 10
12. Ladder

Geldoc_2015-09-03_14hr_09mincpcr_atob_cena.jpg



According to the gel picture colony PCR was unsuccesful. It is possible that none of the colonies contain the right insert.

Miniprepped Amph (no linker) following the kit protocol.
NanoDrop results:

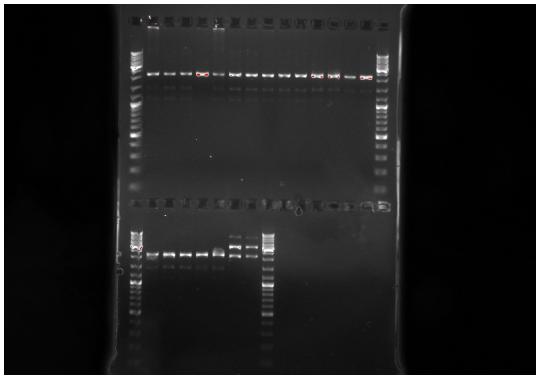
Table1

Sample	DNA (ng/ul)	A260/A280
Amph 11	90,8	1,86
Amph 12	90,7	1,86
Amph 13	81,5	1,86
Amph 14	83,8	1,86
Amph 15	67,8	1,87
Amph 16	77,3	1,88
Amph 17	70,7	1,87
Amph 18	86,9	1,91
Amph 19	79,3	1,89

Restricted Amph 1-19 and GFP+Amph+linker 1 & 2 with PstI following the protocol. However, the incubation was 1,5 h.

Made a 1,3 % agarose gel with EtBr. Restricted Amph, AH043 and GFP+Amph were run in the gel for 40 min with 100 V. Pipeting order was:
I 1. ladder 2 ul 2.-15. Amph 1-14 6 ul 16. ladder 2 ul
II 1. ladder 2 ul 2.-6. Amph 15-19 6 ul 7.-8. GFP+Amph 6 ul 9. ladder 2 ul

Geldoc_2015-09-03_15hr_11min_restAmphGFPAmph.jpg



Result: Because our backbones are biobrick backbones, there should have been only one band in one lane. So it is very likely that our restriction buffer (3.1) or enzyme is contaminated. Of cource, the other possibility is that our cloning has failed. But at least one band for GFP+Amph, there seems to be correct sized part (about 3741 bp)

Transformation of Car Amph (second gibson) was successful (white colonies).
Did o/n cultures of GFP+Amph 1 and 2 and Car Amph 1-9 in 2 ml LB with 2 ul AMP.

4.9.2015

FRIDAY, 9/4

Miniprepped Car Amph (second gibson) following the kit protocol. (NanoDropped on 8.9.)
Stored minipreps in -20 C.

Did fluorescence microscopy samples from o/n cultures of GFP+Amph+linker. Used 1:1000 diluted samples in H2O and undiluted samples. The microscope didn't show properly our samples, and decided that we'll do fluorescence microscopy another day. Samples should have been concentrated in minicentrifuge a little (about 1000-2000 rfc for 1-2 min; not too much or bacteria will become a mush.)